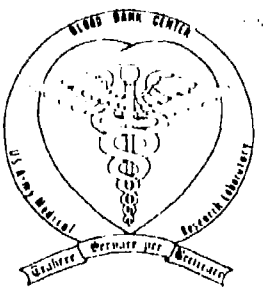
ANTISERA EVALUATION AND OTHER CONSULTATION SERVICES

Brochure



The
*Blood Bank Center Reference Laboratory**

US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121
June 1972

*Accredited by the American Association of Blood Banks, October 1971.

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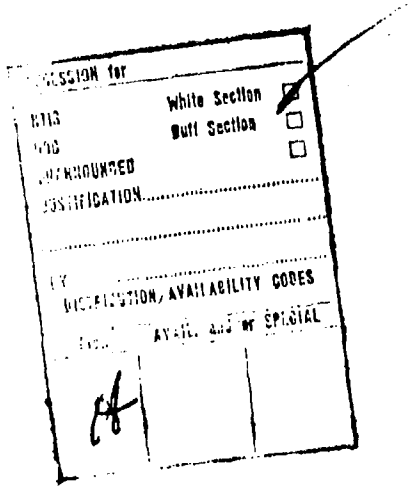

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
US Army Medical Research Laboratory
Fort Knox, Kentucky 40121

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE
ANTISERA EVALUATION AND OTHER SERVICES AVAILABLE IN THE BLOOD BANK CENTER REFERENCE LABORATORY

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Brochure

5. AUTHOR(S) *(First name, middle initial, last name)*
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6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
June 1972	40	None

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 84121689000000	None
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	US Army Medical Research and Development Command, Washington, D. C. 20314

13. ABSTRACT

A brochure has been prepared describing the various quality control tests of blood group reagents and consultation services available at The Blood Bank Center Reference Laboratory. The role of The Blood Bank Center Reference Laboratory in evaluating blood group reagents for the Armed Services is described as well as the interrelationship of this quality control testing with the Defense Medical Materiel Board, the Defense Personnel Support Center, and the Division of Biologics Standards of the National Institutes of Health. Other consultation and testing services include immunohematological studies, forensic studies, Gm testing, and pyrogen testing. A listing of available scientific literature includes 121 laboratory reports, five monographs, and a translation series in blood group immunology.

Details of illustrations in this document may be better studied on microfiche

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Blood Group Reagents						
Quality Control						
Consultation Services						
Immunohematology						
Forensic Studies						
Gm Testing						
Pyrogen Testing						

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ANTISERA EVALUATION AND OTHER CONSULTATION SERVICES

BROCHURE

by

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Blood Bank Center
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

June 1972

MISSION STATEMENTS

Defense Medical Materiel Board: Established by the Secretary of Defense to provide coordination, advice, and assistance on the professional/technical aspects of medical materiel and in the field of medical supply.

Captain R. F. C. MacPherson, MC, USN, Director, Defense Medical Materiel Board

Mrs. Elise N. Hayes, Staff Member, Defense Medical Materiel Board

Defense Personnel Support Center Medical Mission: Procures, stores, stocks, and issues items of medical materiel standardized by the Defense Medical Materiel Board, based on the logistic requirements of the individual medical services.

SUPPORT AGREEMENT

1. The US Army Medical Research Laboratory (USAMRL) agrees to provide services upon written request from the Directorate of Medical Materiel, Defense Personnel Support Center (DPSC), on the following types of items supplied by the receiving activity:

a. Plasma protein fraction.

b. Blood grouping sera.

c. Bromelin, ficin, papain, and trypsin enzyme solutions.

d. Serum, antihuman, Coombs test.

e. Dextran.

f. Albumin normal human serum.

g. Albumin serum reagent, bovine.

h. Globulin, tetanus immune.

i. Globulin, immune serum.

j. Globulin, Rh_0 immune.

k. Other blood derivatives and related products.

l. Pyrogen testing.

m. Blood bags.

2. USAMRL will test other blood related equipment and supplies not described above upon mutual agreement with DPSC.

3. USAMRL will conduct workshop courses (duration: 5 days) for medical materiel inspectors of DPSC.

4. Upon completion of any examination, USAMRL will notify DPSC of any evidence of noncompliance with specifications and/or nonsuitability for issue and use.

Agreement number Z2-A2250F-0001 2, dated 20 September 1971.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Rachel G. Bertram, Cynthia S. Carter, and Dorothy P. Stiglitz for the format, typing, and editing of this monograph.

We also acknowledge the photographic support and technical advice of Richard A. Wheeler, George W. Weeks, James E. Smith, Mary Jo Wyatt, and Philip E. Corbit.

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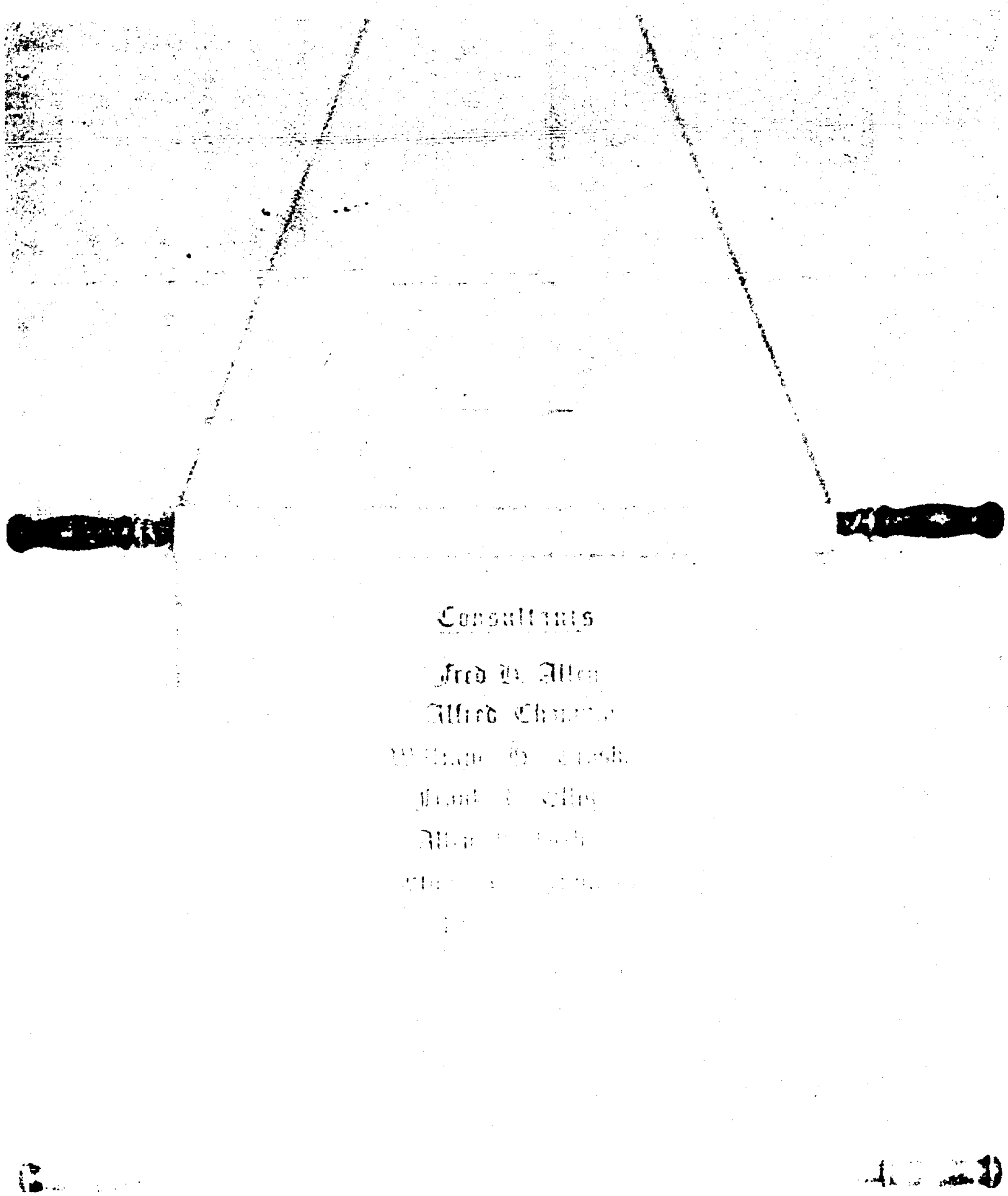

AERIAL VIEW OF USAFTL

BUILDING 1006 - BLOOD BANK CENTER

PROCESSING LABORATORY

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INTRODUCTION

The Blood Bank Center (BBC), US Army Medical Research Laboratory (USAMRL), Fort Knox, Kentucky, operates a reference laboratory. One important function is to evaluate each lot of blood bank antisera purchased on government contract.* The criteria for evaluation are developed from the performance requirements (essential characteristics) which are established by the Defense Medical Materiel Board (DMMB) and incorporated in the purchase description by the Defense Personnel Support Center (DPSC).

Before any laboratory evaluation, a blood bank reagent must conform to the existing minimum requirements established by the Division of Biologics Standards (DBS), National Institutes of Health (NIH). A copy of the NIH release form must accompany the material submitted. Reference standard reagents from the Division of Biologics Standards, National Institutes of Health, are tested in parallel with all blood group reagents submitted to the Blood Bank Center Reference Laboratory for evaluation.

A contract for a particular antiserum is awarded by the Defense Personnel Support Center (DPSC) after the reference laboratory certifies that the antiserum conforms to DPSC specifications. During bottling of any lot, a quality assurance representative from DPSC is present; at this time 12 bottles are selected at random and shipped directly to the BBC Reference Laboratory by him. Six of these samples are tested before shipment is released; the remaining six are stored as reference samples at the laboratory.

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*The following products currently under contract are tested:

1. Anti-A, liquid 5 ml, 6505-159-8475.
2. Anti-A, dried, equivalent to 5 ml, 6505-975-0614.
3. Anti-B, liquid, 5 ml, 6505-159-8500.
4. Anti-B, dried, equivalent to 5 ml, 6505-975-0615.
5. Anti-A,B, dried, equivalent to 5 ml, 6505-935-3998.
6. Anti-A,B, liquid, 5 ml, 6505-584-3038.
7. Anti-Rh_o, liquid, 5 ml, 6505-159-8575.
8. Anti-Rh_o, dried, equivalent to 5 ml, 6505-975-0613.
9. Anti-Rh_orh'rh", liquid, 5 ml, 6505-684-8664.
10. Antihuman, 2 ml, 6505-071-0611.
11. Antihuman, 10 ml, 6505-065-0024.
12. Albumin, bovine, 22%, 10 ml, 6505-890-1639.

The procedures used in testing antiserum are detailed in Annexes A-G.

The DMMB performance requirements for titer, avidity, and specificity for ABO and Rh sera follow:

TABLE 1

ABO Grouping Sera

	Anti-A			Anti-B			Anti-A,B		
		Avidity			Avidity			Avidity	
Cells	Titer	Beginning	Complete	Titer	Beginning	Complete	Titer	Beginning	Complete
A_1	512*	5"	30"				512*	5"	30"
A_2	128	5"	30"				128	5"	30"
A_1B	256	5"	30"	256	5"	30"	256	5"	30"
A_2B	64	45"	3'	256	5"	30"	128	5"	30"
B				512*	5"	30"	512*	5"	30"

*1:256, June 1972.

Antibodies must react with the corresponding antigens only. This is tested by using both serum and saline suspensions of group A, B, O, and AB bloods. The tube and slide methods are used in testing at least ten A's, ten B's, ten O's, and five AB's. A 4+ reaction after centrifugation is required with the tube method.

Tests are performed to insure that no hemolysins and/or nonspecific immune antibodies are present.

TABLE 2

Rh Typing Sera

	Anti-Rh_o			Anti-$Rh_orh'rh"$		
		Avidity			Avidity	
Cells	Titer	Beginning	Complete	Titer	Beginning	Complete
R_1R_1	64	15"	2'			
R_2R_2	64	15"	2'			
R_1R_2	64	15"	2'			
R_1r	64	15"	2'			
R_or				64	15"	2'
r'r				4	30"	2'
r"r				4	30"	2'

Antibodies must react with the corresponding antigens only and are tested with serum suspended cells by the tube and slide methods. In the test tube method at room temperature, the degree of positive agglutination reaction with Rh_0 cells must be ++++ when used according to the directions of the manufacturer. No incubation is permitted. Reading of reaction must be made immediately after spin. At least ten random positive bloods and five negative bloods are used in testing. The anti-Rh_0 must be suitable for testing for the Rh_0 variant $\overline{Rh}_0$ by the indirect Coombs method.

Some of the more important DMMB performance requirements for antihuman sera and bovine albumin follow:

Antihuman Serum

Antihuman serum is tested using the block Coombs titration. The antihuman serum, when diluted 1:16 in saline, must cause agglutination of sensitized Rh_0 positive erythrocytes producing a 1+ reaction, one serial dilution higher than the basic titer of the anti-Rh_0 serum. It must be capable of detecting antibodies in the Rh, Duffy, Kell, Lewis, and Kidd systems and of detecting immune anti-A and immune anti-B in serum by the indirect antiglobulin method.

Using the direct antiglobulin method, the antiserum must detect coated cells from an acquired hemolytic anemia, as well as coated cord cells in cases of mother-child ABO incompatibility. It must detect both gamma and nongamma immunoglobulins.

Specificity of reagent must permit microscopic examination in blood transfusion compatibility testing.

Albumin, Bovine 22%

Albumin, bovine, must be a concentrated 22% (±2%) solution suitable for use in Rh testing, Rh antibody titrations, and compatibility testing. The pH must be between 7.0 and 8.0; the sodium chloride content between 700 and 1,000 mg/100 ml. The albumin solution must not cause hemolysis, crenation, or rouleaux formation of red blood cells.

In addition to testing blood bank reagents, the BBC Reference Laboratory offers the following consultation services to any military installation.

CONSULTATION SERVICES AVAILABLE AT USAMRL

1. Immunohematological studies.
   a. Antibody detection and identification.
   b. Crossmatch problem assistance.
   c. Transfusion reaction studies and assistance.
   d. Screening for rare donors.
2. Forensic studies.
   a. ABO determinations.
      (1) Blood crusts.
      (2) Blood stains.
      (3) Seminal stains.
      (4) Saliva stains.
      (5) Bone.
      (6) Hair.
      (7) Fingernails.
   b. Precipitin testing.
   c. Paternity studies.
   d. Hemoglobin studies.
3. Miscellaneous studies.
   a. Pyrogen studies.
   b. Gm testing (referral).
   c. Special studies upon request.
   d. Hepatitis (Australia antigen) screening.
   e. Analysis of blood bank reagents, purchased through DPSC involved in complaints.
   f. Coagulation studies.

Consultation forms are available upon request. See Annex H for a sample form.

The BBC Reference Laboratory may be reached by telephone:

Day	(502) 624-6656/7051
Night (CQ)	(502) 624-1647
Autovon	464-6656/7051/1647

BLOOD BANK FELLOWS

Blood Bank Fellows (cont)

DPSC WORKSHOP

ANNEX A

PROCEDURE FOR TESTING ANTI-A

1. Titer.

a. Five rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row place 0.7 ml of saline in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.7 ml of anti-A in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the four tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of A_1 cells in the second row, 0.1 ml of a 2% suspension of A_2 cells in the third row, 0.1 ml of a 2% suspension of A_1B cells in the fourth row, and 0.1 ml of a 2% suspension of A_2B cells in the fifth row. Mix. Spin in a serofuge for 30 seconds. Read for agglutination. Let tubes sit at room temperature for 15 minutes and reread.

2. Avidity.

a. Use a 10% suspension of the same cells used in the titer (A_1, A_2, A_1B, and A_2B).

b. Place one drop of the cell suspension on a slide and one drop of anti-A. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually ten random group A bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)

b. Using blood of A_1, A_2, A_1B, A_2B, B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-A are used. Spin immediately. Read for agglutination.

c. These same six blood groups are tested by the stick-tube method giving a serum suspended cell of a 2% suspension in whole blood. Spin immediately. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-A are placed in three tubes. Place one tube at room temperature, one tube at 37 C, and one tube at 4 C for 1 hour. Observe for hemolysis and/or agglutination. Then the three tubes are kept at room temperature for 2 hours. Observe for hemolysis and/or agglutination.

5. Clarity.

a. Liquid antiserum. Material should be clear and free of particulate matter.

b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.

ANNEX B

PROCEDURE FOR TESTING ANTI-B

1. Titer.

a. Four rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row, place 0.7 ml of saline in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.7 ml of anti-B in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the three tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of B_1 cells in the second row, 0.1 ml of a 2% suspension of A_1B cells in the third row, 0.1 ml of a 2% suspension of A_2B cells in the fourth row. Mix. Spin in a serofuge for 30 seconds. Read for agglutination. Set tubes aside for 15 minutes at room temperature and reread.

2. Avidity.

a. Use a 10% suspension of the same cells used in the titer (B, A_1B, and A_2B).

b. Place one drop of the cell suspension on a slide and one drop of anti-B. Mix. Observe for beginning and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually ten random group B bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)

b. Using blood from A_1, A_2, A_1B, A_2B, B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-B are used. Spin immediately. Read for agglutination.

c. These same six blood groups are tested by the stick-tube method, giving a serum suspended cell of a 2% suspension in whole blood. Spin. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-B are placed in three tubes. Place one tube at room temperature, one at 37 C, and one at 4 C for 1 hour. Observe for hemolysis and/or agglutination. Keep the three tubes at room temperature for 2 hours. Observe for hemolysis and/or agglutination.

5. Clarity.

a. Liquid antiserum. Material should be clear and free of particulate matter.

b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.

ANNEX C

PROCEDURE FOR TESTING ANTI-A,B

Cells needed: A_1, A_2, B, A_1B, and A_2B - 2% suspensions in saline.

1. Titer.

a. Six rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row place 0.8 ml of saline in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.8 ml of anti-A,B in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the five tubes in the back and 0.8 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of A_1 cells in the second row, 0.1 ml of a 2% suspension of A_2 cells in the third row, 0.1 ml of a 2% suspension of B cells in the fourth row, 0.1 ml of a 2% suspension of A_1B cells in the fifth row, and 0.1 ml of a 2% suspension of A_2B cells in the sixth row. Mix. Spin in serofuge for 30 seconds. Read for agglutination. Let tubes sit at room temperature for 15 minutes and reread without spinning.

2. Avidity.

a. Use a 10% saline suspension of same cells used in the titer (A_1, A_2, B, A_1B, and A_2B).

b. Place one drop of the cell suspension on a slide and one drop of anti-A,B. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually 10-15 random group A, B, and AB bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)

b. Using blood of A_1, A_2, B, A_1B, A_2B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-A,B are used. Spin immediately. Read for agglutination.

c. The same six blood groups are tested by the stick-tube method giving a serum suspended cell of a 2% suspension in whole blood. Spin immediately. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-A,B are placed in three tubes. Place one tube at room temperature, one tube at 37 C, and one tube at 4 C for 1 hour. Observe for hemolysis and/or agglutination. Then set aside the three tubes for 2 hours at room temperature. Observe for hemolysis and/or agglutination.

5. Clarity.

a. Liquid antiserum. Material should be clear and free of particulate matter.

b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.

ANNEX D

PROCEDURE FOR TESTING ANTI-Rh_0

Cells needed: Group O, R_1r, R_1R_1, R_1R_2, R_2R_2 - 2% suspensions in 22% albumin.

1. Titer.

a. Five rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row place 0.7 ml of group AB serum in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.7 ml of anti-Rh_0 in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the four tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of R_1r cells in the second row, 0.1 ml of a 2% suspension of R_1R_1 cells in the third row, 0.1 ml of a 2% suspension of R_1R_2 cells in the fourth row, and 0.1 ml of a 2% suspension of R_2R_2 cells in the fifth row. Mix. Incubate at 37 C for 1 hour. Mix. Spin in a serofuge for 45 seconds. Read for agglutination.

2. Avidity.

a. Use as whole blood the same cells used in the titer (R_1r, R_1R_1, R_1R_2, and R_2R_2).

b. Place two drops of the cell suspension on a slide (heated to 37 C), add one drop of anti-Rh_0. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually ten random Rh positive and five Rh negative bloods are tested by the slide and stick-tube methods.

b. Test, by slide and stick-tube methods, using cells of R_1r, R_1R_1, R_1R_2, and R_2R_2 in the appropriate suspensions.

c. Using known Rh_o positive and negative cells, test for this Rh variant. Place two drops of anti-Rh_o in a tube, two drops of 22% albumin in a second tube (negative control). Add one drop of a 2% suspension of cells to each tube. Incubate at 37 C for 30 minutes. Wash three times, add two drops of Coombs, spin and read.

4. Clarity. Material should be clear and free of particulate matter.

ANNEX E

PROCEDURE FOR TESTING ANTI-Rh_0rh'rh"

Cells needed: Group O, R_0r, r'r, r"r - 2% suspension in 22% albumin.

1. Titer.

a. Four rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row place 0.6 ml of group AB serum in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.6 ml of anti-Rh_0rh'rh" in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the three tubes in the back and 0.6 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of R_0r cells in the second row, 0.1 ml of a 2% suspension of r'r cells in the third row, and 0.1 ml of a 2% suspension of r"r cells in the fourth row. Mix. Incubate at 37 C for 1 hour. Mix. Spin in a serofuge for 45 seconds. Read for agglutination.

2. Avidity.

a. Use as whole blood the same cells used in the titer (R_0r, r'r, and r"r).

b. Place two drops of the cell suspension on a slide (heated to 37 37 C), add one drop of anti-Rh_0rh'rh". Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually ten random Rh positive and five Rh negative bloods are tested by the slide and stick-tube methods.

b. Test by slide and stick-tube methods, using cells of R_0r, r'r, and r"r in the appropriate suspensions.

4. Clarity. Material should be clear and free of particulate matter.

ANNEX F

PROCEDURE FOR TESTING ANTIHUMAN SERUM

1. Materials.

a. Red blood cells. For more reactive tests, use homozygous Rh_o positive cells preferably of genotype R_2R_2. If these are not available use genotype R_1R_1 or R_1R_2. Reasonably fresh cells should be used.

b. Anti-Rh_o antiserum. Use anti-Rh_o antiserum which has a minimum titer of 1:32.

2. Quantitative test procedure.

Sensitization of cells with dilutions of anti-Rh_o.

a. Wash cells in normal saline once. After washing, there should be a minimum packed cell volume of 0.8 ml.

b. Make a 2% cell suspension.

c. Place 5.0 ml of the 2% cell suspension into each of six test tubes (use graduated centrifuge tubes, if possible). Label the tubes 1:16, 1:32, 1:64, 1:128, 1:256, 1:512.

d. Add more normal saline to each tube and centrifuge. After centrifugation, each tube should have a 0.1 ml packed cell volume. Aspirate all of the saline from each tube.

e. While the cells are being centrifuged, the dilutions of the anti-Rh_o antiserum can be made. Label six 13 x 100 mm test tubes 1:16, 1:32, 1:64, 1:128, 1:256, 1:512.

f. Place 12.0 ml normal saline in tube labeled 1:16, place 6.0 ml normal saline in the remaining tubes.

g. Add 0.8 ml anti-Rh_o to tube 1:16. Rinse the pipette in the tube several times. This gives a dilution of 1:16 anti-Rh_o in a total volume of 12.8 ml.

h. Make twofold dilutions in the remaining tubes by removing 6.0 ml from tube 1:16 into tube 1:32, and so on. Discard the remaining 6.0 ml of the 1:512 dilution.

i. Place 4.9 ml of each dilution of anti-Rh_o into the appropriately labeled tube containing 0.1 ml packed cells. Mix well to resuspend cells.

j. Incubate at 37 C for 1 hour.

k. Wash four times with normal saline. This is important because insufficient washing may cause a false negative reaction.

l. Add 4.9 ml normal saline and resuspend packed cells.

m. Place 0.1 ml of sensitized cells in six tubes from each dilution of anti-Rh_o, a total of 36 tubes. Add 0.1 ml of antihuman (Coombs) serum, using undiluted 1:2, 1:4, 1:8, 1:16, and 1:32 dilutions. (See Diagram #3. Titration of Antihuman Serum, Appendix A, Minimum Requirements: Antihuman Serum for the Antiglobulin Test, NIH.)

n. Centrifuge and read. (See 3.5 potency requirements, Minimum Requirements: Antihuman Serum for the Antiglobulin Test, NIH.)

3. Qualitative test procedure.

a. Place two drops of Rh_o positive cells (2% cell suspension) in a 12 x 75 mm test tube.

b. Add two drops of 1:16 dilution of anti-Rh_o.

c. Spin and read. (Test should be negative; if positive, make a higher dilution of anti-Rh_o.)

d. Incubate at 37 C for 1 hour.

e. Spin and read. (Test should still be negative.)

f. Wash four times with saline and decant completely after last wash.

g. Add two drops of antihuman serum. Centrifuge and read. (This should be positive.)

4. Potency testing (using a known positive antigen-antibody system):

a. Depending on the titer of the antisera used, make either a 1:10 or a 1:20 dilution of anti-rh', anti-rh", anti-hr', and anti-hr".

b. Place two drops of each dilution into a test tube.

c. Add two drops of a 2% cell suspension of the corresponding Rh antigen.

d. Incubate at 37 C for 30 minutes.

e. Wash four times with normal saline and decant completely after last wash.

f. Add antihuman serum, spin, and read. (Tests should be positive.)

g. Repeat, using undiluted anti-K, anti-Jk^a, anti-Le^a, and anti-Fy^a antisera with two drops of a 2% cell suspension of their corresponding antigens. (Tests should be positive.)

h. Test for immune anti-A and anti-B, using group O serum (previously known to have immune A and B) according to the AABB screening method. (See pages 59 and 60 of AABB Manual, 5th edition.)

BLOCK COOMBS TITRATION

Coombs Dilution	Dilution of Anti-Rh_o Sensitized Cells (Group O)					
	1:16	1:32	1:64	1:128	1:256	1:512
Undiluted						
1:2						
1:4						
1:8						
1:16						
1:32						

Dilution for Coombs	Amount of Saline	Amount of Coombs
1:2	0.4 ml	0.4 ml
1:4	0.6 ml	0.2 ml
1:8	0.7 ml	0.1 ml
1:16	0.75 ml	0.05 ml
1:32	1.55 ml	0.05 ml

5. Anticomplement activity (C'4 and C'3).

a. Materials.

(1) Five percent dextrose in water.

(2) Isotonic saline.

(3) Fresh clotted blood (less than 24 hours old), clot and serum separated.

(4) Liquid K_2 EDTA, 5 mg per drop.

(5) Parafilm.

(5) Five Pasteur pipettes.

b. Procedure.

(1) Mark three 13 x 100 mm tubes at the 2 ml level and number them 1, 2, and 3.

(2) Transfer 5% dextrose in water to tubes 1 and 2 and fill to the 2 ml level.

(3) Transfer isotonic saline to tube 3 and fill to 2 ml level.

(4) Add three drops of liquid EDTA to tube 2.

(5) Add five drops of fresh serum to each tube.

(6) Cover tubes with parafilm and mix several times.

(7) Add three drops of fresh whole clotted blood (from the same donor as the serum) to each tube.

(8) Cover tubes with parafilm and mix well.

(9) Incubate all three tubes for 10 minutes at 37 C.

(10) Label three 10 x 75 mm tubes 1, 2, and 3.

(11) Place two drops of the mixed cell suspensions from the larger tubes into the appropriately numbered small tubes.

(12) Wash the cells three times in saline.

(13) Add one drop antiglobulin reagent, serofuge, and read.

RECORD RESULTS

Cell	Low Ionic Strength		Normal Ionic Strength
	1 Complement Coated	2 Complement Blocked	3 No Coating
Antiglobulin Test			

ANNEX G

PROCEDURE FOR TESTING BOVINE ALBUMIN

Cells needed: Group O, R_1r, R_1R_1, R_1R_2, R_2R_2.

1. Test for hemolysis, crenation, and rouleaux formation of red blood cells.

   a. Place two drops of albumin into several tubes.

   b. Add two drops of a 2% suspension of group O cells to each tube.

   c. Observe macroscopically and microscopically for crenation of cells, hemolysis, and rouleaux formation. None should be present.

2. Test for clot formation in crossmatching procedure.

   a. Place two drops of plasma in tube.

   b. Add one drop of a 2% suspension of cells (obtained from segment on bag).

   c. Add two drops of albumin.

   d. Incubate at 37 C for 30 minutes, spin, and read. Observe closely for clot formation. Test should be negative.

3. Test for observing hemolysis.

   a. Place 0.1 ml of serum (known to have a hemolysin) in a tube.

   b. Add 0.1 ml of a 2% suspension of A_1 or B cells.

   c. Add 0.1 ml of albumin.

   d. Incubate at 37 C for 1 hour.

   e. Spin and read. Hemolysin should be present.

4. Quantitative testing.

   a. Make serial dilutions of a previously tested anti-Rh_o in group AB serum.

   b. Make 2% cell suspensions of group O, R_1r, R_1R_1, R_1R_2, and R_2R_2 cells in the albumin.

c. Add 0.1 ml of the cell suspension to 0.1 ml of the anti-Rh_0 dilution.

d. Incubate at 37 C for 1 hour.

e. Mix. Spin for 45 seconds and read. Titer should be the same as when previously tested.

5. Sodium chloride content.

a. Determine the chloride content.

b. Sodium chloride content may then be determined by using this formula:

mEq Cl/l x 5.85 = mg NaCl/100 ml

c. Should be between 700-1000 mg/100 ml.

6. pH determination. pH of the albumin should be between 7.0 and 8.0.

7. Percent of albumin. Albumin content should be 22% ± 2%.

ANNEX H

Blood Bank Center
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

REQUEST FOR CONSULTATION

Send Report To: Date:_____

Name:_____

Hospital:_____

Street:_____

City & State:_____ Zip Code: _____

Telephone No:_____ Area Code: _____

Send specimen to: Reference Laboratory
Blood Bank Center
US Army Medical Research Laboratory
Fort Knox, Kentucky 40121

Procedure for submitting samples:

1. Send freshly drawn samples, clearly labeled with full name and date.

2. Send 15 to 20 ml clotted blood and 5 ml anticoagulated blood. SEPARATE MOST OF SERUM FROM CLOT

3. Send specimens AIR MAIL, SPECIAL DELIVERY, and label "Blood specimen - refrigerate as soon as possible." Mail container to arrive at Reference Laboratory between Monday and Friday, if possible.

4. Notify the Reference Laboratory by telephone of the shipment.
Autovon: 464-6656
Commercial: 624-6656, Area Code 502

INFORMATION CONCERNING CASE

1. Patient's name_____Serial No._____

Sex_____Age_____Race_____

Diagnosis_____

2. ALL FEMALE PATIENTS:

Number of pregnancies_____Any difficulty?_____

Number of exchanges, if necessary_____

3. ALL PATIENTS:

Number of transfusions and dates_____

_____

Number of group O (universal donor) units received_____

Type of reaction and number of units received_____

_____

Estimate number of units needed_____

4. DIFFICULTY ENCOUNTERED:

A. Crossmatch problem_____

1. Saline_____ 2. Albumin_____

3. Coombs_____ 4. Enzyme_____

No. of donors compatible_____ No. of donors incompatible_____

Is patient receiving any drugs?_____List drugs_____

_____

B. Antibody identification_____

1. Saline_____ 2. Albumin_____

3. Coombs_____ 4. Enzyme_____

C. Hemolytic Disease of the Newborn_____

D. Other (explain in detail)_____

_____

_____

_____

_____

ANNEX I

AVAILABLE SCIENTIFIC LITERATURE

US ARMY MEDICAL RESEARCH LABORATORY REPORTS

Report Number	Title
671	Blood Components - Their Preparation and Use
677	Interaction of Progesterone and Aldosterone With Red Blood Cells of the Rat
678	Military Blood Banking - Identification of the Group O Universal Donor for Transfusion of A, B, and AB Recipients - An Enigma of Two Decades
707	Screening Procedures Employing Semiautomated and Fully Automated Technics
708	Effect of Immunizations on Blood Group Antibody Production
717	Inhibitory Properties of Serum Proteins on the Enzymatic Sequence Leading to Lysis of Red Blood Cells by Snake Venom
719	Comparison Studies of Whole Blood Stored in ACD and CPD and With Adenine
735	The Effect of Sulfhydryl Reagents on the Binding of Human Hemoglobin to Haptoglobin
739	Testing of Blood Grouping Cards Under Field-Type Conditions
741	Survival of ACD Blood After Serial Storage
747	The Glomerular Filtration of Hemoglobin: A Proposed Mechanism
752	Rate of Hemolysis of Fresh and Stored Human Red Blood Cells and the Effect of Progesterone
755	Clinical Hematological Values, Erythrocytic Indices and Osmograms of *Cercocebus torquatus atys* and *Papio anubis*
756	A Continuous Body Temperature Monitoring System for Utilization in Pyrogen Testing
758	Stored Whole Blood after Long Distance Transportation

Report Number	Title
762	Investigation of Materials and Methods for Air Delivery of Whole Blood and Blood Products
777	Subunit Dissociation of Certain Abnormal Human Hemoglobins
781	Long-term Preservation of Biologicals for the Forensic Laboratory and Their Areas of Application
785	An Adaptation of the Peters and Van Slyke Method for Measuring Whole Blood Oxygen Dissociation Equilibria
790	Hemoglobin Function in Stored Blood
792	Studies on Stored Liquid Whole Blood. II. Use of Packed Red Cells
794	*In Vivo* and *In Vitro* Studies on BME Hemoglobin
798	Demonstration of Blood Group Substance A Bound to *Pasteurella pestis*
799	Preservation of Human Blood from Male and Female Donors
803	Automated Detection of Gm Factors of Human γG Globulin
804	Human Serum Antiglobulins and Immunization
805	Evaluation of Automated Multichannel Blood Grouping Apparatus. II. Comparison with Manual and Dried Reagent Methods
806	Evaluation of Automated Multichannel Blood Grouping Apparatus. I. Procedure and Reagent Standardization for Blood Grouping
807	The Occurrence of Blood Group Substances A and B in Proprietary Gamma Globulin of Placental Origin
808	Clinical Evaluation of Transfused Blood after Long-term Storage in ACD with Adenine
809	Effects of Environmental Temperature on Selected Blood Shipping Containers
810	Comparison of Autologous and Nonautologous Transfusions of ACD-Adenine Blood

Report Number	Title
815	Serum Agglutinators Reacting with Pepsin Treated Gamma Globulin: I. "Naturally Occurring" Reactants in the Serum of Subhuman Primates
816	Study of Military Blood Banking and Crossmatching Using Blood Group Antigens Stored over Five Months in ACD-Adenine
818	ABO Antibodies. I. Methods for Quantification of ABO Hemolysins and Soluble Blood Group Substances A and B
826	Studies on Stored Liquid Whole Blood. I. Effect of Volume Transfused on *In Vivo* Survival Measurement
828	Evaluation of Automated Multichannel Blood Grouping Apparatus. III. Studies on Detection of Human Hemagglutinins
830	Evaluation of an Automated Method for Blood Grouping in the Military Service - A System Analysis
833	Studies on Stored Liquid Whole Blood. III. Evaluation of Plastic Collection Containers
834	A Semiautomated Method for Quantitative Fibrinogen Determinations
836	The Hemoglobin Function of Blood Stored at 4°C
837	The Therapy of Experimental Hemorrhagic Shock with Red Cell Stroma Free Hemoglobin Solution
838	Evaluation of Stroma Free Hemoglobin Solution: Effects on Renal Function in Cynomolgus Monkeys
839	The Effect of Methylene Blue Addition to Whole Blood During Prolonged Storage
840	Relative Viscosity and Specific Gravity of Human Blood During Cold Storage
842	Blood Shipping Boxes Evaluated Under Varying Modes of Heat Exposure
843	Isolation and Initial Studies on a Proteinase from Human Erythrocyte Membranes
844	Changes in Human Erythrocyte Membrane Proteins During Storage

Report Number	Title
845	Investigation of Nephrotoxic Effects of Adenine and Its Metabolic Product, 2,8-Dioxyadenine, on Primates (*Macaca irus*)
851	The Role of Automated Blood Grouping as an Information Retrieval System
852	A Single Card Laboratory Reference Index System
854	A Consideration of Some Correlates of Fainting in Blood Donors
855	A Comparative Study of the Incidence of Blood Donor "Reactors" in Smokers and Nonsmokers
856	The Control of Hemoglobin Function in Blood Stored for Transfusion Purposes
858	A Fail-Safe Approach to Incompatible Blood Transfusions
863	A Biphasic Extraction for 2,8-Dioxyadenine
865	A Quality Control Approach to Improved Donor Care
867	Standardization of Blood Transfusion Reaction Studies in the Military. Delegation of Responsibility for a Medical Team Concept. Role of the Hospital Transfusion Board
868	Evaluation of Human Plasma After Prolonged Storage in Plastic Containers
870	The Clotting System of Monkeys: A Comparison of Coagulation Factors and Tests between Cynomolgus Monkeys (*Macaca irus*) and Humans
875	Biochemical and Physical Properties of Stored Male and Female Donor Blood
876	Effect of Plasma Removal on Blood Stored in ACD With Adenine
877	The Hemoglobin Function and 2,3-DPG Levels of Blood Stored at 4°C in ACD and CPD: The pH Effect
878	Hemoglobin Function in Stored Blood: IV. Red Cell Viability and Function in ACD and CPD With Adenine and Inosine
880	Tissue Transplantation - The Universal Donor and Blood Group Antibodies

Report Number	Title
881	Biological Alterations Occurring During Red Cell Preservation
882	Studies on Stored Liquid Whole Blood. IV. Effects of Temperature and Mechanical Agitation
887	Effect of Heparinized Saline Infusion and Hypotension on Calcium Homeostasis in the Dog
891	Saliva Agglutinins and Automated Methods for Universal Donor Screening
892	A Practical Synopsis of Consumption Coagulopathy
893	The Murayama Test. Part I: Evidence for the Modified Murayama Hypothesis for the Molecular Mechanism of Sickling
894	The Murayama Test. Part II: Principles, Technique, Interpretation, and Data
895	Sickling Reversed and Blocked by Urea in Invert Sugar: Optical and Electron Microscopy Evidence
896	Sickle Cell Crisis Terminated by Use of Urea in Invert Sugar in Two Cases
897	Modified Sickledex Tube Test: A Specific Test for S Hemoglobin
898	An Automated Screening Method for the Specific Detection of Homozygous and Heterozygous S Hemoglobin
900	Hemolysis and Intravascular Coagulation Due to Incompatible Red Cell Transfusion in Isoimmunized Monkeys
902	Disseminated Intravascular Coagulation and Renal Failure: Production in the Monkey With Autologous Red Cell Stroma
909	Steroid Hormones in the Preservation of Human Blood
910	Embryonic, Fetal, and Neonatal Hemoglobin Synthesis: Relationship to Abortion and Thalassemia
912	Consumption Coagulopathy: Practical Principles of Diagnosis and Management
914	The Function of Human Hemoglobin: Salt Effects

Report Number	Title
915	Hemoglobin Function in Stored Blood: VI. The Effect of Phosphate on Red Cell ATP and 2,3-DPG
916	Platelet Contamination of Erythrocyte Membrane Preparations
918	Cargo Coding Developments in Military Blood Bank Logistics
924	Hemoglobin Function in Stored Blood: Effects of Salts and Glutathione
925	Hemoglobin Function in Stored Blood: Further Effects of Phosphate on Red Cell ATP and 2,3-DPG
926	Blood Preservation Solutions: A Review
927	The Salivary Anti-A and Anti-B Isoantibody System in Group O Males
929	Plasma Transfusion Reactions in Isoimmunized Monkeys
930	Turbidity Measurements of Solubilized Human Erythrocyte Membranes
931	Military Blood Banking (Civil Disasters)
932	Hemoglobin Function in Stored Blood: IX. A Modified Preservative with Optimal pH to Maintain Red Cell 2,3-DPG (Function) and ATP (Viability)
933	Forensic Aspects of Transfusion Reactions
934	Comparison of Blood Collected by Vacuum Methods and Gravity Flow
935	Changes in Erythrocyte Membranes during Cold Storage II
936	The Detection of Sickle Cell Disease in Large Human Populations by an Automated Technique
937	The Forensic Testing Laboratory, 1971--Problems, Progress, and People
938	Physicochemical Changes in Erythrocyte Membranes During Cold Storage in the Presence of Progesterone
939	Effect of Varying Concentrations of Adenine, Inosine, and Methylene Blue on the Useful Storage Life of Blood

Report Number	Title
942	Dithionite Tube Test - A Rapid, Inexpensive Technique for the Detection of Hemoglobin S and Non-S Sickling Hemoglobin
943	Automated Dithionite Test for the Rapid, Inexpensive Detection of Hemoglobin S and Non-S Sickling Hemoglobinopathies
944	The Murayama Test for Hemoglobin S (A Simplification in Technique)
945	Sickledex Test for S Hemoglobin: A Critique
955	Automated Quantitation of A and B Blood Group Substances
958	Blood Component Logistics
960	Hemolytic, Coagulant, and Renal Effects of Transfused IgG and IgM Derived from Plasma of Isoimmunized Monkeys
961	Ability of Rabbit IgG Fab′ Fragment Specific for a Human Species Antigen to Block Reactivity of HL-A Antisera
962	Specificity of a Rabbit Antihuman Lymphocyte Serum
963	Passive Suppression Characteristics of a Rabbit Antihuman Lymphocyte Serum
964	Management in Military Blood Banking for Conservation of Blood Resources: New Aspects Concerning the Blood Donor Base
965	Mass Screening of Military Populations for Hemoglobin S by the Automated Dithionite Test
966	A Collected Bibliography of Clinical Advances in Sickle Cell Disease Based on the Murayama Molecular Hypothesis
969	Pulmonary Hemorrhage Syndrome as a Manifestation of Disseminated Intravascular Coagulation: Analysis of 10 Cases
972	Thermal Destruction of Anti-A_1 and Anti-$A(A_2)$ from Group O and Group B Serum
973	Electrocardiographic and Respiratory Changes Observed in Blood Donors During Phlebotomy
974	Hemoglobin Function in Stored Blood: XII. Effects of Varying Phosphate Concentrations on Red Cell ATP and 2,3-DPG with Adenine and Inosine

Report Number	Title
975	Blood Preservation Solutions. XI: Raising the pH to Improve Hemoglobin Function
978	Urea, Urease, Cyanate, and the Sickling of Hemoglobin S
979	The Effects of Platelets on the Storage Properties of Human Erythrocytes
980	Sickle Cell Disease: Clinical Advances by the Murayama Molecular Hypothesis
Monograph	Pitfalls of Blood Grouping and Pretransfusion Tests (Library of Congress Catalog Card Number 75-606639)
Monograph	Genetics for the Reference and Forensic Testing Laboratory (Library of Congress Catalog Card Number 77-175026)
Monograph	Military Blood Banking 1941-1971. Lessons Learned Applicable to Civil Disasters and Other Considerations (Library of Congress Catalog Card Number 78-184862)
Monograph	Immunohematology (Library of Congress Catalog Card Number 77-175027)
Monograph	Blood Group Immunology: Translation and Reproduction of Early Scientific Treatises (Library of Congress Catalog Card Number 76-188448)
Brochure	Antisera Evaluation and Other Consultation Services Available at The Blood Bank Center Reference Laboratory
Translation Series	
	Gammelgaard, Arne. On Rare, Weak A Antigens (A_3, A_4, A_5, and A_X) in Man (Library of Congress Catalog Card Number 64-65449)
	Hartman, Grethe. Group Antigens in Human Organs (Library of Congress Catalog Card Number 71-606638)
	Selected Contributions to the Literature of Blood Groups and Immunology: (Library of Congress Catalog Card Number 71-606638)
	Volume I. The ABO System (Dunsford Memorial)

Volume II.	Secretion of Blood Group Substances and Lewis System	
Volume III.	Part I.	Constitutional Serology and Blood Group Research
	Part II.	M, N, and P Systems
Volume IV.	Part 1.	Anthropologic Data
	Part II.	Blood Groups and Their Areas of Application
Volume V.	Landsteiner Centennial	

ANNEX J

MISCELLANEOUS PHOTOGRAPHS

ACTIVATION AND GROWTH OF BLOOD PROGRAMS
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

*1964	Staff Study
1965	Blood Transfusion Research Division
1965	Blood Group Reference Laboratory
**1965	Quality Control Monitoring (DPSC)
1965	Blood Bank Fellowship Program (3 Fellows) Army
1966	Medical Corps Officer Training Program
1966	Reference and Forensic Testing Laboratory
1966	Blood Transfusion Division
1967	Institutional Membership, AABB
***1967	Approved Institution of Training AABB-ASCP
1969	Blood Coagulation Laboratory
1969	Transfusion Reaction Model
1969	Blood Components Center
1969	Blood Bank Fellowship (4 Fellows) 3 Army, 1 Navy
1970	Histocompatibility (Lymphocyte Typing) Laboratory
1970	Field Testing Laboratory
1970	311-F1 Blood Bank Training for Enlisted Personnel
1971	Blood Bank Center
1971	Blood Research Division
1971	AABB Reference Laboratory
1971	Blood Bank Fellowship (5 Fellows) 3 Army, 1 Navy, 1 Air Force

FUTURE GOALS

Frozen Red Blood Cell Bank
Rare Donor Registry

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*Crosby & Camp
**Defense Personnel Support Center
American Association of Blood Banks
***American Society of Clinical Pathology

US ARMY
BLOOD TRANSFUSION RESEARCH STAFF 1965-1971
FORT KNOX, KENTUCKY


CONTE, N. F.
1969-71


CAMP, F. R., JR.
1965-71


SHIELDS, C. E.
1965-70


DAUBER, L. G.
1966-68


REED, L. J.
1966-68


BUNN, H. F.
1966-68


KAPLAN, H. S.
1967-69


LITWIN, S. D.
1967-69


DAWSON, R. B., JR.
1968-71


LOPAS, H.
1968-71


BIRNDORF, N. I.
1968-71

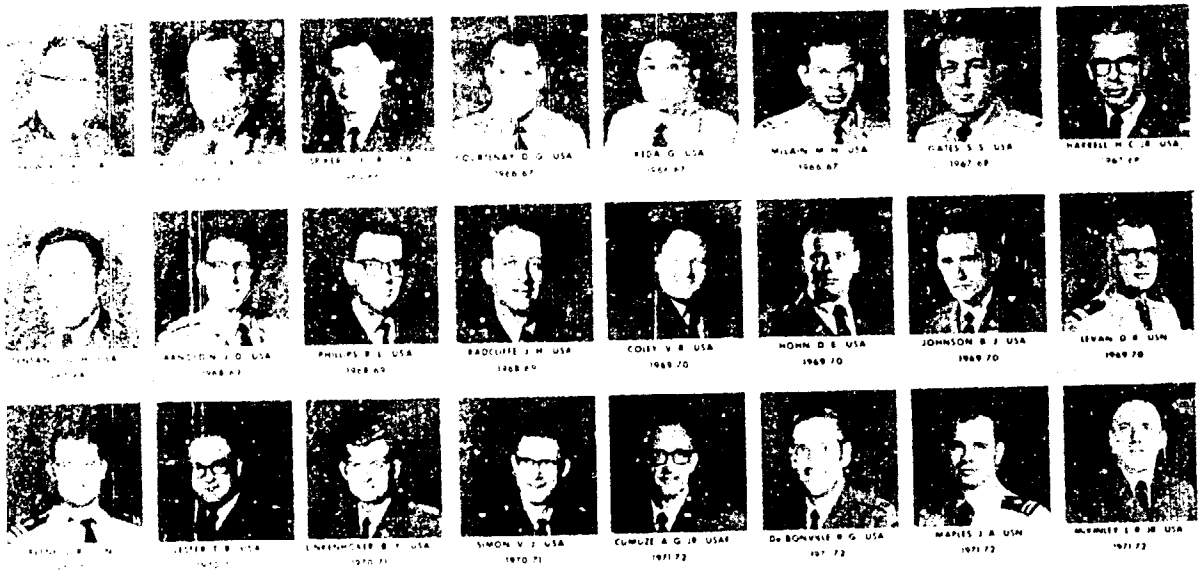

BELL, C. E., JR.
1968-71

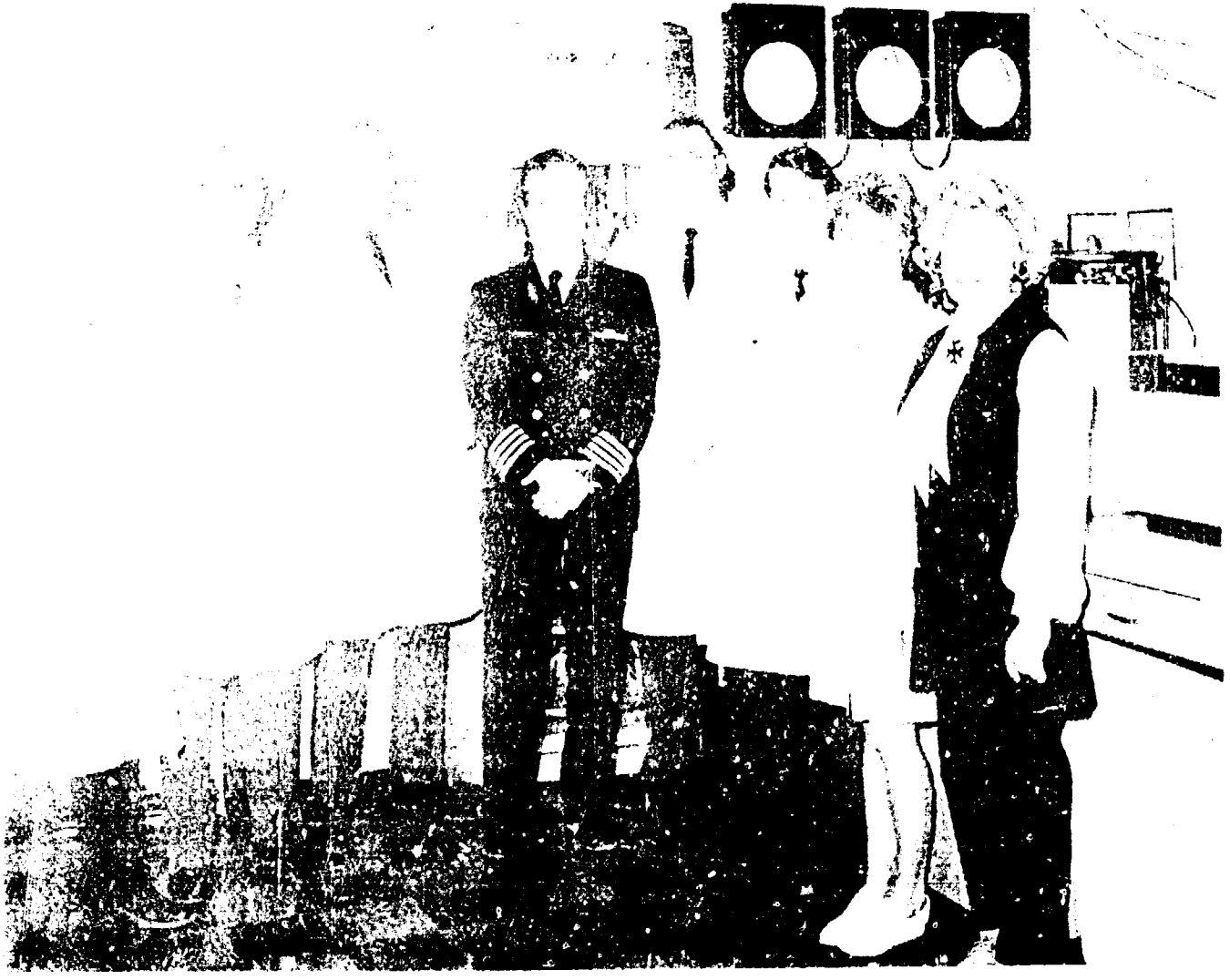
CONSULTANTS, ADVISORS AND FRIENDS

US ARMY BLOOD BANK FELLOWSHIP PROGRAM

WALTER REED ARMY INSTITUTE OF RESEARCH
WASHINGTON, D. C.
1958 - 1965

US ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY





Left to right: J. A. Maples, A. G. Cumuze, Jr., R. G. DeBonville, R. F. C. MacPherson, L. R. McKinley, Jr., J. H. Young, Margaret E. McPeak, and Elise N. Hayes.

Left to right: Ima G. Shirley, Lillian W. Necessary, R. F. C. MacPherson, Margaret E. McPeak, F. R. Camp, Jr., and Elise N. Hayes.

Left to right: Margaret E. McPeak, Elise N. Hayes, and R. F. C. MacPherson.